A Symposium on

THE CHEMICAL BASIS OF HEREDITY

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DR. LEDERBERG: An optimistic report of this symposium might indicate a new era in genetic study, where factorial descriptions are (about to be) replaced by chemical ones. The details of the speculations which have been put forward here may all prove to be wrong, but we are at a stage in the development of chemical genetics where we need stepping stones as well as foundation blocks. Furthermore, most of the experimental reports have been designedly tentative, and their authors assure us they are aware of the gaps which have to be filled. It may therefore be inappropriate to question them too closely in critique.

Some of the residual issues are so obvious that they perhaps ought to be stated. On the chemical side:

1. What is the detailed structure of DNA? No one appears to have taken serious exception to the Watson-Crick model for the basic plan, but many microscopic deviations have been suggested, and may be needed to explain singularities of genetic behavior such as variable mutation rates. Is pairing always perfect? Are there interactions between adjacent nucleotides in the same chain? Are there segmental interruptions in the chains? Are there linkages other than phosphate-3-5-diester bonds? Having achieved the general plan, what are the prospects of a complete analysis of sequence of bases? We lack precise methods of end-group analysis of polynucleotides, and more work in sugar chemistry and enzymology may be needed to find them. And if we had the chemical methods, what would we analyze? Levinthal gave us the only plausible answer here, the large "piece" in the phage nucleus: any other material now evident, and perhaps this too, would certainly be hopelessly heterogeneous. Until we have real information along these lines, we cannot pretend to know the chemical basis of genetic differences, however plausible the guesses of the moment may appear.

2. How is DNA synthesized? Kornberg and his associates have made such startling progress in the elaboration of an in vitro system that one is tempted to call a moratorium on other speculation. They have emphasized the urgency of knowing whether the polymer is a biologically specific product. The requirement for a DNA primer and for all four deoxynucleoside triphosphates hints that it is, but we must still learn whether the synthesis is a replication or a random lengthening of the polynucleotide primer.

3. Tests of biological specificity of nucleic acids. These are sadly rare. For RNA we have hardly more than the infectivity of tobacco mosaic virus, either as the native RNA, or reconstituted with a protective protein. For DNA we have the pneumococcal and Hemophilus transforming systems. The problems involved in quantitative assay for biological activity with the pneumococcus have been amplified by Hotchkiss and Ephrussi-Taylor. Goodgal's report suggests that the Hemophilus system may be in some respects easier, but this also needs to be studied more extensively. At the present time we have no method

of assaying the biological activity, hence specificity, of extracted DNA from phage or from other sources. A number of other claims of DNA-mediated transduction (transformation) may be found in the literature, but none of them has yet stood up well enough to serve the purpose. It would be highly desirable to work out a DNA-transductional system in an enteric bacterium (e.g., Salmonella or *E. coli*) because of the technical facility and background knowledge of genetic work with these organisms. The reports of Boivin, which are often quoted, unfortunately cannot be verified owing to the loss of his cultures at the time of his death. Perhaps the protoplast systems mentioned by Spiegelman may serve as analytical tools for the specificity both of DNA in heredity) and RNA (in functional development).

Until such systems are further refined, we may have to call our symposia "Chemistry and Heredity" rather than "Chemistry of Heredity."

Dr. Chargaff indicated the very real language barrier, and the trouble it can raise at a symposium of genetics, chemists and crystallographers. He may have been interested to learn that this is a problem even among the geneticists, to the point where 'gene' is no longer a useful term in exact discourse. It should not be surprising that (some) geneticists are remarkably preoccupied with semantic problems. The series of constructs involved in genetic analysis are not necessarily more abstract than those used, e.g., in structural analysis of organic compounds, but they are usually expressed verbally rather than mathematically. Because the words are part (I hope not all) of the tools of the trade, the geneticist has to try to keep them sharp. One of the outstanding grinding wheels here was Benzer, who with "muton," "recon" and "cistron" has fabricated some euphonious and utilitarian contributions to our language. I don't know whether he meant them any more seriously than the Anglo-Saxonized "mit," "rit" and "pfit," but whether he did or not, we are going to find it difficult to avoid talking about cistrons from now on. Unfortunately, as I eavesdropped to catch notes for this discussion, I heard variant uses, and we can only beg that our colleagues adhere scrupulously to the new dictionary: a distron is not defined in respect to any enzyme or other purported gene product, but is a group of mutants whose functional relationship is inferred from their common membership in a cis-trans position-effect group.

One danger in postulational terminology is to confuse the word for a fact. An important contribution from Benzer was his demonstration that his mutants could be grouped into *unique* cistrons. No other case of position effect has been so intensively studied, and it would be premature to hold that this is a general description of patterns of position effect, and therefore that position effect must be interpreted in terms of common primary function. The well-known effect of transposed heterochromatin in Drosophila, and McClintock's findings (reviewed by Rhoades at this symposium) in maize, speak for another

mechanism of position effect: disturbances at one point of a chromosome may spread some distance down its length. Depending on the overlap of these spreading effects, of three mutants, *a*, *b*, and *c*, *a* and *b* might be found to show position-effect, as well as *b* and *c*, but *a* and *c* might not. The *a*-*b* and *b*-*c* cistrons are therefore not unique, but overlap on *b*. This mechanism makes no demand of functional identity of the mutants. Only further study can tell how generally position effects may be understood from these two, or from other, viewpoints.

The term *gene* represented the hope that the chromosome could be considered as an array of discrete units, whose irreducibility would be confirmed by mutational, recombinational, and functional tests. Finer analysis and current theories of DNA structure, if translatable to the chromosome, suggest a more monotonous continuum which is not segmented into natural unit "genes." A mutation is therefore a local change on a chromosome, not necessarily in a "gene." Rhoades showed that many mutations are disqualified as "point mutations." Their existence is not a semantic fancy. A point mutation is most concretely defined as one which results from the substitution or deletion of a single nucleotide. It remains to be seen whether any mutations actually arise in this way. One helpful criterion might come from the prediction that at any (recombinational) point, no more than four (or including deletion, five) alternative configurations would be possible, one for each base. Benzer's studies represent the most encouraging approach to this kind of demonstration. Until the finer structure is cleared up, there is bound to be nomenclatural confusion. I had hoped that such terms as *locus* and *allele* might be reserved to the traditional and (to my own mind) more objective concept of recombinational units, but there is no agreement on this point today. Perhaps we should adopt Levinthal's suggestion that all discussion of terminology be proscribed, but that each author prescribe his own use of the terms. The topic is belabored only because of the discordance in current literature.

Language problems are not confined to genetics. I was struck by several references to "*mere* exchange reactions"; I thought I understood what "exchange reaction" meant, but I didn't understand the "*mere*." Is it true that a macromolecule can exchange an interstitial monomer and keep its biological specificity? If so, it might help to understand the incorporation step of transductional genetics, wherein a fragment presumably must exchange with an organized chromosome.

DR. SPIEGELMAN: I should like to say a few words about the exchange problem. It seems to me unwise to accept without caution conclusions about the protein synthesizing mechanism which are derived solely from incorporation data. I think it unlikely that a labeled amino acid can be inserted in the middle of a fully-formed protein molecule in solution. The act of incorporation is therefore probably not trivial and related to the protein-synthesizing mechanism

There remains, however, an element of uncertainty which makes it difficult to interpret incorporation data unambiguously. I have already noted in my discussion the experiments of Gale and Folkes and their relation to the interpretation of unequal labeling of a protein molecule.

There are three types of auxiliary experiments, the performance of which could help in assaying the significance of incorporation experiments:

(1) The effect of homologous and heterologous amino acid analogues on the extent and rate of incorporation.

(2) A comparison of incorporation in the presence and absence of supplementation by a complete mixture of amino acids.

(3) Evidence that the labeled amino acid is being inserted all along the protein molecule in α-peptide linkages.

DR. HOAGLAND: Let me add to Dr. Spiegelman's discussion the following comments in clarification of the meaning of so-called amino acid "exchange" reactions and their significance in protein synthesis, since much work has been done in our and other laboratories on the assumption that the incorporation of C^{14} labelled amino acids into protein is indicative of protein biosynthesis. It would appear to be highly unlikely that an amino acid could exchange with an already formed and completed protein, and Gale has suggested that the anomalous exchange he observes may be due to interaction of the amino acid with some pre-protein on a template. Furthermore, the possibility of glutathione synthesis as an explanation for glutamic acid "exchange" in Gale's system must be considered.

We have used the word "incorporation" to describe the *irreversible* incorporation of C^{14} amino acids into protein which we and others are studying in mammalian tissues. In these reactions, both in vivo and in vitro, the addition of C^{12} amino acids after an initial incorporation of C^{14} amino acids results in no loss of previously incorporated amino acid. Furthermore, this process is dependent, in vitro, upon ATP for energy, a soluble protein fraction containing amino acid activating enzymes, small amounts of GDP or GTP, and intact ribonucleoprotein particles. When the protein so labelled is isolated and subjected to all procedures known to remove any non-peptide bound amino acids, the radioactivity remains. The radioactive amino acids are released from the protein on hydrolysis at the same rate as the corresponding C^{12} amino acid. Small peptides which contain the labelled amino acid have been isolated from the hydrolysate and identified. The labelled amino acid is found throughout the protein and not just in terminal positions. We believe for these reasons that incorporation is very likely a measure of protein biosynthesis.